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Phytochemical analysis, Thin Layer Chromatography and Gas Chromatography Mass Spectroscopy profile of *colocasia esculenta* and *manihot esculanta* extracts and their potential for drug discovery

Ismail Rabiu[1*], Muhammad Yusha'u[2], Jaafaru Isah Abdullahi[3], Abdulazeez Muhammed[4]

ABSTRACT

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Over the years, these herbal remedies obtained from medicinal plants have proven to be a remarkable source of newer and more potent therapeutic agents and have therefore taken the central stage in most research centers in the world. The aim of this research is to analyzed *Colocassia esculenta* and *Manihot escalanta* plants for the presence of the bioactive compounds and as well assess their potential for drug discovery. Fresh Leaves of the two plants were collected from Kaduna Nigeria and Phytochemical analysis, Thin Layer Chromatography (TLC), and Gas Chromatography-Mass Spectroscopy (GCMS) were carried out on the aqueous and methanolic extracts of the two leaves. The presence of tannins, flavonoids, alkaloids, phenols, and saponins was observed in all the extracts. TLC gave four and five separate bands for the aqueous and Methanol extracts respectively with Rf values ranging from 0.12 to 0.90. Among the two plants, *M. esculenta* extracts were identified via GCMS as having the highest number of different compounds (35) with *C. esculenta* having 26. The results obtained in this study revealed the extracts as having different bioactive agents capable of having inhibitory activity thereby showcasing the possibility of better chemotherapeutic outcomes in the treatment of bacterial, fungal, and viral infections.

Keywords: *Colocasia esculenta, Manihot esculanta*, Medicinal plants, Gas Chromatography-Mass Spectroscopy, Thin Layer Chromatography

1. INTRODUCTION

The use of medicinal plants as prophylactic agents has been in existence for centuries. Research estimates suggest that approximately 25% of all modern medicines were produced from medicinal plants. Among the many factors that promote the use of phytomedicines are their easy-to-get nature, and lack of side effects, among others. It is thought that among the major causes of the growing level of antibiotic resistance among microbes is the indiscriminate use of antibiotics (Adamu et al., 2021). As the need for safe and effective drugs continues to grow, the use of plants with medicinal properties to manage many diseases is becoming more attractive mainly due to the continual emergence of multidrug-resistant enzymes such as the Carbapenamases, and Extended Spectrum beta-lactamases which continue to render inactive the many antibiotics that were otherwise effective in the treatment of specific diseases (Rawayau et al., 2022; Bale et al., 2021; Rabiu et al., 2022; Alagbe et al., 2023).

The World Health Organisation estimates that as much as 80% of the global population relies heavily on either medicinal plants or their products to manage their various health complications. Therefore, screening many other plants scientifically for the presence of bioactive compounds is necessary with a view to finding the bases for their use traditionally. *Manihot esculenta* and *Colocasia esculenta* belong to the Euphorbiaceous and Araceous family, respectively. The plants are cultivated mainly in the subtropical and tropical regions of the world, mainly as a food crop. The plant leaves concoctions are used traditionally to manage different diseases (Geetha et al., 2014; Rabiu et al., 2022). The leaves and bark are traditionally used to treat skin infections, diarrhea, wound, stomach, and other disorders. Several published literatures have reported the presence of diverse groups of bioactive compounds which are known to possess inhibitory activity against a diverse group of microorganisms (Alzabt and Rukayadi, 2021; Al-Kaf et al., 2019; Meena et al., 2011).

This study aims to carry out qualitative Phytochemical analysis, Thin Layer chromatography (TLC), and Gas Chromatography-Mass Spectroscopy (GCMS) profiles of *C. esculenta,* and *M. esculanta* leaves, thereby assessing their potential for drug discovery. *Colocasia esculenta* (CE) Linn. (Family: Araceae) is commonly called Taro (English), and 'Gwaza' in (Hausa). Traditionally, the plant (Figure 1) is used in the management of complications such as asthma, wound infections, internal hemorrhage, diarrhea, neurological problems, Arthritis, and skin infections caused by different pathogens such as *S. aureus, E. coli,* Enterobacter, and Salmonella spp. among others (Bhagyashree et al., 2011; Eleazu et al., 2013). Fresh leaves are known to be an exceptional source of Minerals, proteins, dietary fiber, and vitamins. These includes amino acids, lipids, carotenoids, luteolin, riboflavin, apigenin, calcium, Vitamin C, phosphorous, oxalic, acid, niacin, and iron (Dutta and Aich, 2017).

Figure 1 Whole plant, leaves and tubers of *C. esculenta*

Phytochemically, *C. esculenta* was reported to contain flavones, Saponins, sterols, tannins, apigenin, luteolin, phenolics, and anthocyanins among others (Dutta and Aich, 2017; Al-Kaf et al., 2019). *M. esculanta* is commonly called cassava (English) and 'Rogo' (Hausa). In West Africa and Nigeria, research has not been extensively carried out on the biochemical properties of *M. esculenta,* its tuber as well as its medicinal properties. Research findings have indicated an activity of the leaves (Figure 2) extract against a diverse group of pathogenic microbes covering both bacteria, viruses, and fungal isolates. This previous research has not focused on the

identification of the individual component of this plant that might be responsible for the activity (Geetha et al., 2014; McCallum et al., 2017).

Figure 2 Whole plant, leaves and tubers of *M. esculenta*

The leaves and bark are traditionally used to treat diarrhea, wound, stomach, and other skin infections. Asides from the plant's nutritional benefits, at a given proportion, the tuber peels, oils extracted from the seed, and the leaves are traditionally used to prepare concoctions to treat diseases such as diarrhea, dysentery, skin infections, and other animal diseases (Ehiobu and Ogu, 2018; Thiyagarajan et al., 2010; Santos-Silva et al., 2021). More so, the tuber peels and the seed which contains oil have a wide range of inhibitory activity against diverse fungal isolates and pathogenic bacteria covering both Gram positive and Gram-negative isolates (Ehiobu and Ogu, 2018; Mustarichie et al., 2020).

2. METHODOLOGY

Study Area

This study was carried out at Bayero University, Kano State, the North-western part of Nigeria. Kano is located within latitude 12oN and 13oN and longitude 8oE and 9oE (Figure 3). To the South-east, Kano State shares borders with Bauchi State, to the south-west, it shares borders with Kaduna State, and Jigawa and Katsina State to the Northeast and North-west, respectively (National population commission (NPC), 2006).

Plant Sample Collection, Authentication, and Preparation

Fresh *Colocasia esculenta* and *Manihot esculanta* leaves were collected from Kaduna, Kano, and the Bauchi States. The plant was authenticated by the chief Herbarium, at the Plant Biology Department BUK, and a Voucher specimen was deposited. The leaves were processed as described by (Bale et al., 2021). The leaves were rinsed in clean running water, air dried at room temperature, and ground and later sieved.

Plant Extracts Preparation

Extraction of the plant phytoconstituents was carried out using water and methanol as separate solvents as described by (Sofowora and Suganthi, 2017).

Aqueous Extraction

The Percolation extraction method was employed where 100g of the powdered plant leaves were transferred into 500ml distilled water and kept in a mechanical shaker for 14 days. This was carried out according to the method conducted by (Rabiu et al., 2022). Using a rotary evaporator, the filtrate was concentrated at a temperature of 600C.

Figure 3 Map of Kano State showing the study site and other states with which Kano state shares boundaries (National population commission (NPC), 2006).

Methanolic Extraction

Using methanol, the powdered plant leaves were extracted by Soxhlet extraction. A Hundred (100g) of the powdered plant leaves was dispensed into a clean muslin cloth and inserted into the Soxhlet tube. About 500ml of methanol was dispensed into the Soxhlet thimble after which the Soxhlet extractor was set up. The heating mantle was set at a working temperature of 35-40oC for 12 hours. The extracts were evaporated using a rotary evaporator at a working temperature of 450C

Qualitative Phytochemical Analysis

Both the Aqueous and Methanol extracts were subjected to qualitative phytochemical analysis using standard phytochemical methods as described by (Sofowora and Suganthi, 2017).

Test for Alkaloids (Dragendoff's test)

Each extract was weighed (0.1ml) differently in a test tube and Dragendoff's reagent (2 drops each) was added. A Positive result was denoted by the appearance of orange-red precipitate with turbidity.

Test for Glycosides (Fehling's test)

Each extracts were weighed (1ml) in a separate test tube, and 10ml of 50% H_2SO_4 was added. After 15mins heating, Fehling's solution (10ml) was added, followed by heating. The appearance of brick red precipitates indicates a positive result.

Test for Tannins (Ferric chloride test)

To every 2ml of the extracts, 2ml of water was added. Two (2) drops of 5% ferric chloride ($FeCl_3$) solution was added. The positive test was inferred by the appearance of blue, black, or green colour.

Test for Flavonoids (Hydrogen chloride test)

Magnesium ribbon was added to 4mg/ml of each of the extracts and then a drop of concentrated HCL was added. A positive test was inferred by the appearance of red colour.

Test for Saponins (Frothing test)

To every 0.5g of the extracts dispensed, 5ml of water was added, followed by vigorous shaking. Positive result was observed following the appearance of Persistent froth.

Phenol test (Phenol test)

A gram of each extract was spotted on a filter paper followed by the addition of phoshomolybdic acid reagent (single drop). The spotted extract on the filter paper was exposed to ammonia vapours. A Positive result was observed following the appearance of blue colour on the spot.

Test for steroids (Liebermann-Burchardt test)

One (1) ml of each of the extract and that of chloroform was mixed and 2ml of acetic anhydride was added. Two (2) drops of concentrated sulphuric acid were added again and the presence of dark green coloration indicates a positive result.

Test for Anthraquinones (Born Trager's test)

Ten (10) mg of each of the extracts was mixed with 0.2ml of each concentrated hydrochloric acid and 10% ferric chloride solution. Following cooling and filtration, diethyl ether was added and shaken vigorously. Strong ammonia was used to extract the ether. The presence of red colour in the aqueous layer was considered positive for anthraquinones.

Thin layer Chromatography

The method described by Demetrio et al., (2016) was employed.

Preparative Thin Layer Chromatography

Preparative thin layer chromatography (TLC) was conducted on both the methanol and aqueous extracts in order to obtain the best solvent system that can fractionate the extracts in the main analytical TLC. An already industrially coated (aluminium sheets: 20×20cm) TLC plates (Merck KGaA Darmstadt, Germany) were used and various solvent systems were tried from high polar, moderately polar to low polar combinations. Chloroform, methanol and water ratios: 70:20:10, 60:30:10, and chloroform and methanol were tried at ratios: 60:40, 65:35, 70:30, 80:20, 85:15, 90:10, and 95:5. Lastly N-hexane and Ethyl acetate were tried at ratios: 40:60, 45:55, 30:70 and 70:30. However, N-hexane and Ethyl acetate at the ratio of 70:30 was employed as it gave better separation for both the aqueous and methanol extracts.

Analytical Thin Layer Chromatography

Industrially coated thin layer chromatography plates (TLC) (Merck KGaA Darmstadt, Germany) were used. The solvent system obtained above was used in the analytical TLC. Using fine capillary tubes, 1% of methanolic solutions of standards and investigated extracts were spotted on the TLC plates. Forty spots were spotted separately and allowed to dry. The developing chamber was used to place the spotted plate end down while ensuring the solvent level is strictly below the spots. As the solvent continue to rise by capillary action in the adsorbent, it was ensured that the solvent front do not exceed the marked line which is about 1cm to the top end of the adsorbent. Following the removal of the plate, the position of the solvent front was marked. The plates were visualized under ultraviolet light of 254nm wavelength. For more visibility, the plates were further visualised in 10% H2SO4, and all the bands were visibly observed and all observed colours were compared with that of the standard to know the compound. The retention factor (*Rf*) value was calculated using the formula:

$$Rf = \frac{\textit{distance traveled by the sample from the point of application to the center of spot}}{\textit{distance travelled by solvent}}$$

Gas Chromatography-Mass Spectrophotometry (GCMS) Analysis

The method described by Demetrio et al., (2016) was adopted. An electron ionization system with ionization energy of 70 eV was used, and the Ultrapure helium gas was maintained at a constant flow rate of 1 mL/min, while the oven was maintained at a temperature of 50 to 150∘C at a rate of 3∘C /min, followed by maintaining it at an isothermal condition for 10 min. The working injector temperature was set at 230∘C, 250∘C, and 290∘C, respectively. Using ethanol, the extracts were diluted at a ratio of 1/100 and 1 μL was introduced in the split mode. Using a solvent delay of 120secs, the mass spectra were maintained at a range of 45–450 m/z. For the identification of the individual components of the extracts, their mass spectra and retention time was matched with that of the National Institute of Standard and Technology (NIST) (Pawar et al., 2022; Bhusari et al., 2020). This allows for the complete identification of each component of the test extracts.

Statistical Analysis

The results obtained were subjected to two-way ANOVA (SPSS version 26) (to analyse the variation between the yields of the two plants.

3. RESULTS AND DISCUSSION

The *M. esculanta* extracts (Aqueous 26.7%; Methanol 13.3%) were observed to have more yield as compared to that of *C. esculenta* (Aqueous 16.0%; Methanol 8.2%). All the extracts were observed to have a gummy texture and chocking smell, with the methanol extracts having a greenish black colour while the aqueous was having a reddish-brown colour. Table 1 presents the results of these findings. Having observed a significant difference in the yield of the two plant extracts ($P<0.05$). The results of the *C. esculenta* extracts differ from that of Al-Kaf et al., (2019) who recorded a higher yield in the methanol extracts (29%) of *C. esculenta* as compared with the aqueous extracts (26%). In the *M. esculanta* extracts, slight variation exists, upon comparison with the findings of Rasha et al., (2014) who recorded a percentage yield of (16.36%) using ethanol as solvent of extraction. The variation might be a result of the varying quantity of the bioactive compounds (phytochemicals) present in these plants as detected in the work of (Yusha'u, 2011).

Table 1 The Physical Properties of *C. esculenta* and *M. esculanta* extracts

Plant	Plant extracted	Solvent used	(%) Yield	Odour	Colour	Texture
C. esculenta	100g	Methanol	8.2	Chocking smell	Greenish black	Gummy
	100g	Aqueous	16.0	Chocking smell	Reddish brown	Gummy
M. esculanta	100g	Methanol	13.3	Chocking smell	Greenish black	Gummy
	100g	Aqueous	26.7	Chocking smell	Reddish brown	Gummy

The difference in the polarity of the solvents of extraction might as well contribute to variation in the extracts yield, with Methanol having a relative polarity of 0.762 and 1.000 for water (Placeholder1), even though there is no significant difference (P>0.05). In the phytochemical analyses result (Table 2), all the methanol extracts were observed to contain (flavonoids, Saponins, tannins, glycosides, alkaloids, and phenols) with the exception of anthraquinones in the methanol extracts of *C. esculenta* and steroids in *M. esculanta.* However, the aqueous extracts of *M. esculanta* was observed to contain all the tested Phytoconstituents (flavonoids, Saponins, tannins, glycosides, alkaloids, steroids, anthraquinones and phenols), while the *C. esculenta* aqueous extracts do not contain glycosides and anthraquinones. In all the extracts of *C. esculenta*, anthraquinones was observed not to be present. These metabolites are known to exerts different antiviral, antifungal and antibacterial activity.

Table 2 Phytochemical Constituents of plants extracts

Phytoconstituents	**Test**	***C. esculenta* extracts**		***M. esculanta* extracts**	
		Methanol	**Aqueous**	**Methanol**	**Aqueous**
Flavonoids	Shinoda test	+	+	+	+
Saponins	Frothing/Foam test	+	+	+	+
Tannins	Braemer's test	+	+	+	+
Glycosides	Fehling's test	+	-	+	+
Alkaloids	Dragendoff's test	+	+	+	+
Steroids	Lieberma Burchardt test	+	+	-	+
Anthraquinones	Born Trager's test	-	-	+	+
Phenols	Phenol test	+	+	+	+

Keys: (+) = Present, (-) = Absent

In specific, the activity of many ethno-medicinal plants, have been linked with the presence of flavonoids (Rabiu et al., 2022; Kumari et al., 2018; Arjun, 2022; Bharti and Arora, 2011). Tannins are reported to possess pharmacological properties mainly because of their astringent activity, which precipitates proteins, protects tissue, and improve wound healing (Bale et al., 2021). Alkaloids play a key role in the body's defense mechanisms. They are known for their metabolic activities and constitute more than 20% of the entire plant's metabolites. For their ability to interfere with cell division, along with other properties confer on them antimicrobial properties. Furthermore, alkaloids are known as anti-inflammatory agents, cardioprotective, and anaesthetics. They are specifically used as nicotine, ephedrine, and quinine are all known preparations of alkaloids used clinically (Yusha'u, 2011). Flavonoids are known to exhibit strong antiviral, antioxidant, and anticancer properties (Mariyappan et al., 2011).

They also confer cardio-protective and neuroprotective effects. Saponins are also known to exhibit anticancer properties, neutralise drug poisoning and acute lead poisoning, lower blood glucose levels, decrease blood lipids and confer protection against hypercholesterolemia. Diets containing high levels pf saponins can inhibit platelet aggregation and dental caries (Bale et al., 2021; Yusha'u, 2011). The result of the *C. esculenta* extracts is in conformity with the findings of Al-Kaf et al., (2019) but varies with that of Nakade et al., (2013), who recorded negative for Flavonoids using the Alkali reagent test. While that of *M. esculanta* differs from that of Rasha et al., (2014) Who did not observe the presence of flavonoids, glycosides, and alkaloids using the lead acetate test, Bromine water test, and Mayer's test respectively while using ethanol as a solvent for extraction.

However, similar results were observed with the present findings as steroids were observed to be absent in the Methanol extracts but present in the aqueous extracts. The dissolving ability of the solvent for extraction into a specific type of solution might contribute to the variation of the phytochemicals present in the various extracts (Kawo et al., 2011). More so, the use of a given solvent and specific extraction method, variations in geographical conditions, and the extraction of a given part of the plant are identified to perform an important role in the variation of plant's phytoconstituents. For the Thin Layer Chromatography (Table 3). The methanol extracts were observed to have more bands (5 in each extracts) compared with the aqueous extracts having 4. The weight of all the recovered extracts was within (3.2mg to 5.9mg) following, washing, filtration, and drying.

Table 3 Thin Layer Chromatography of the investigated extracts showing the R*f* values and colour of spots (Solvent system ratio: 70%:30% for N-hexane and Ethyl acetate, respectively)

Plant extract	Separated Bands	*Rf* values	Weight of scrapped bands (mg)	Weight of extracts recovered from the bands (mg)	Colour under (10% H2SO4)	Colour (under 254nm)	Suspected Compound/group
***C. esculenta* aqueous**	4	0.37	11.3	4.3	Light ash	Dark ash	-
	4	0.48	12.0	4.8	Light yellow	Deep yellow	Flavonoids
	4	0.59	10.5	5.2	Light green	Dark green	Phenolics
	4	0.75	13.0	5.9	Brown	Dark brown	Rutin
***C. esculenta* methanol**	5	0.26	12.6	4.9	Deep yellow	Deep yellow	Flavonoids
	5	0.35	10.9	5.1	Light green	Dark green	Phenolics
	5	0.44	11.8	4.7	Brown	Brown	Rutin
	5	0.61	10.4	4.1	Light Ash	Dark ash	-
	5	0.72	10.3	5.1	Black	Ash	-
***M. esculanta* aqueous**	4	0.60	13.5	5.4	Light yellow	Deep yellow	Flavonoids
	4	0.90	12.9	5.5	Light ash	Dark ash	-
	4	0.12	10.2	4.0	Light green	Dark green	Phenolics
	4	0.16	11.0	5.3	Brown	Brown	Rutin
***M. esculanta* methanol**	5	0.16	12.5	4.8	Light yellow	Deep yellow	Flavonoids
	5	0.25	11.8	4.1	Light green	Dark green	Phenolics
	5	0.37	12.4	4.8	Black	Ash	-
	5	0.57	11.8	3.9	Dark brown	Dark brown	Rutin
	5	0.71	12.3	3.2	Creamy	Yellow	-

Key: *Rf* = Retention factor, (-) = Absent

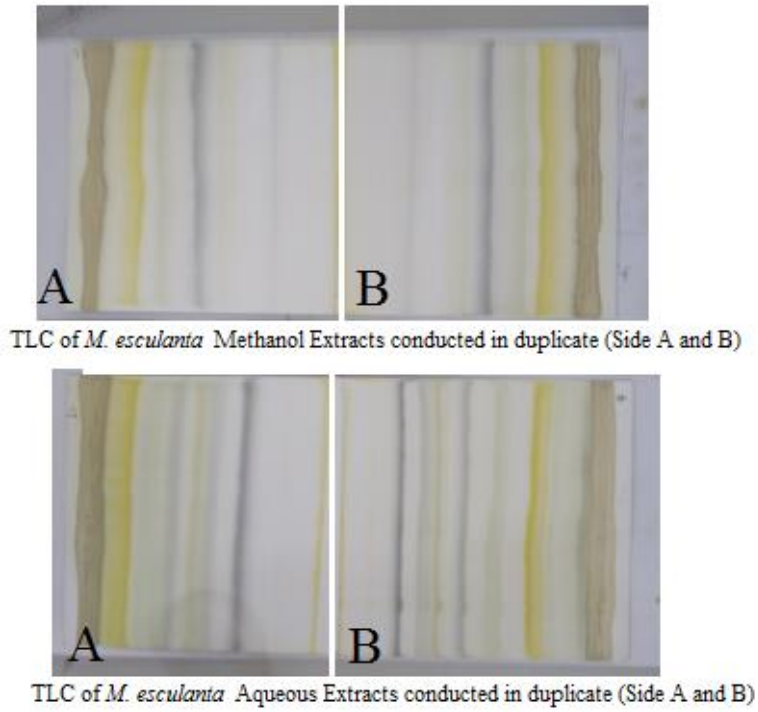

Figure 4 TLC analysis of *M. esculanta* extracts (Aqueous and Methanol)

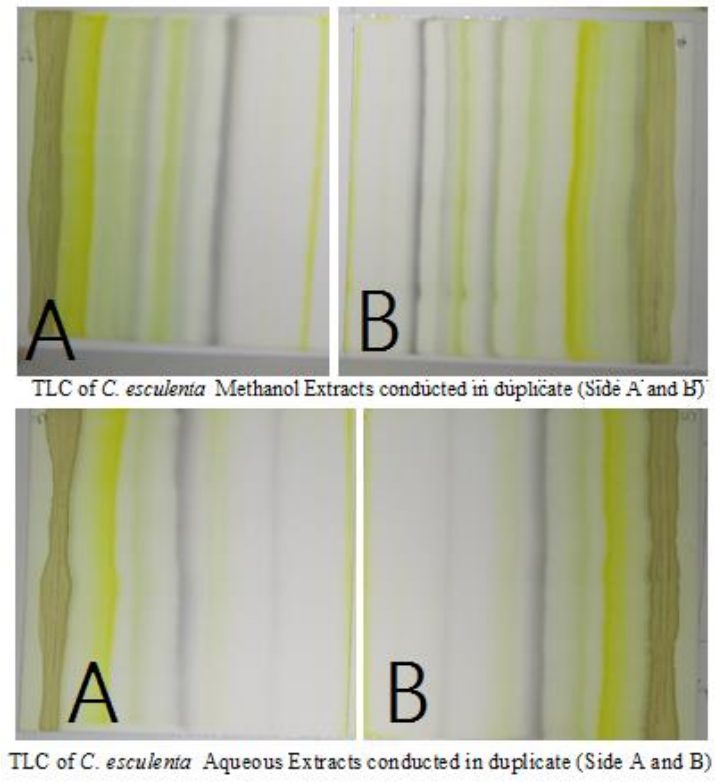

Figure 5 TLC analysis of *C. esculenta C. esculenta* (Aqueous and Methanol)

The TLC results revealed the presence of different compounds in the plant extracts with different R*f* values at a solvent system ratio of N-hexane 70% and Ethyl acetate 30%. Flavonoids, phenolic, and Rutin were detected in all the extracts appearing in different colours (Figure 4 and 5). Varying quantities of bands were recovered, with Rutin and phenolics having the highest quantity in the aqueous and methanol extracts of *C. esculenta* extracts respectively. In the *M. esculanta* extracts, rutin and flavonoids were observed to have the highest recovery. Following the GCMS analysis of all the active (methanol) extracts (Tables 4 and 5), they were identified as having 26 and 35 different compounds for *C. esculenta* and *M. esculanta* respectively, separated by their various peak numbers. The results shows, 2-3-dihydroxypropyl ester (peak number 12, 17, 21, 23, 24, 25, and 26); 9,12-Octadecadienoic acid (Peak 1, and 5); 2-hydroxy-1-(hydroxymethyl) ethyl ester (Peak 18 and 22) and Palmitoleic acid (peak 2) as the major constituents of the *C. esculenta* extracts (Figure 6, 7, 8, 9, 10 and 11), while *M. esculanta* extracts were observed to have 9,12-Octadecadienal (Peak 14, 15, 16, 33, 34 and 35); Hexadecanoic acid (Peak 6, 8, 11, 12 and 28); 9-Oxabicyclo[6.1.0] nonane (Peak 17 and 20) and Oleic Acid (Peak 23 and 29) as the most abundant component (Figure 12, 13 and 14).

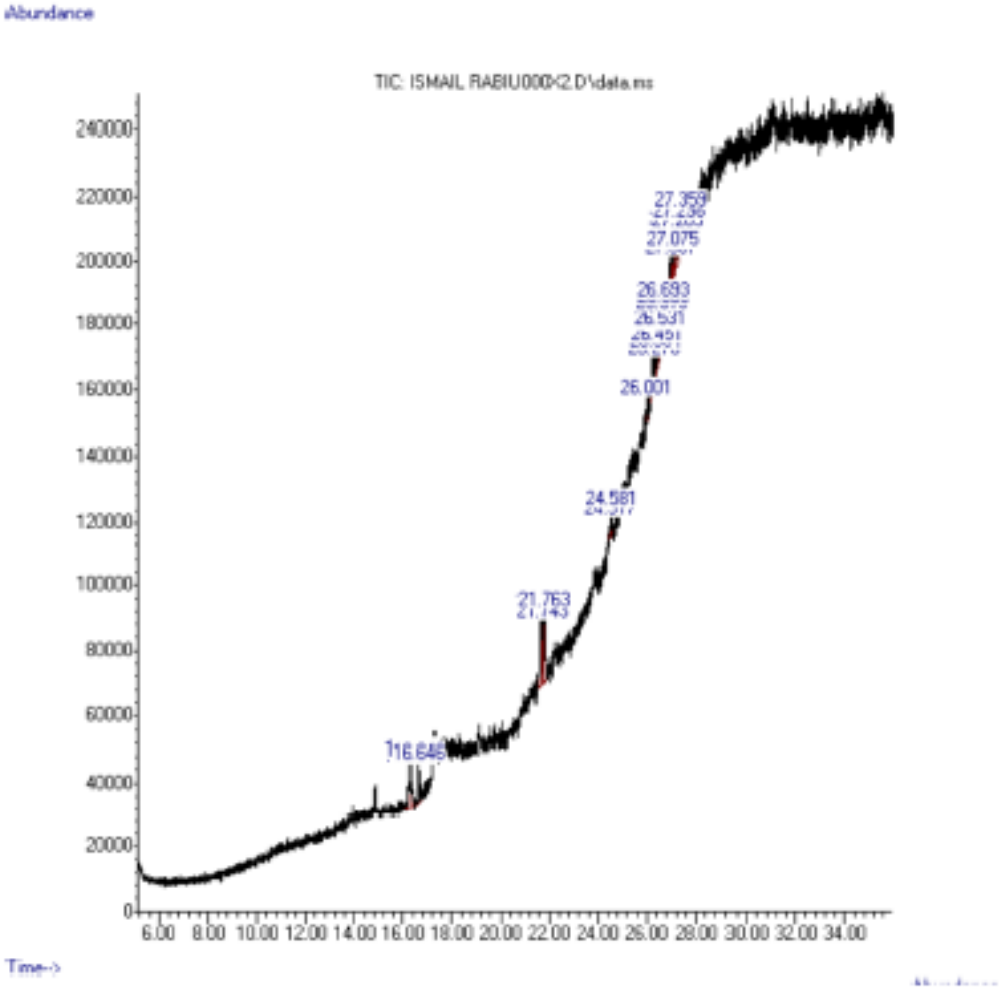

Figure 6 GCMS Chromatogram of *C. esculenta* extracts (Methanol) showing the various phytoconstituents

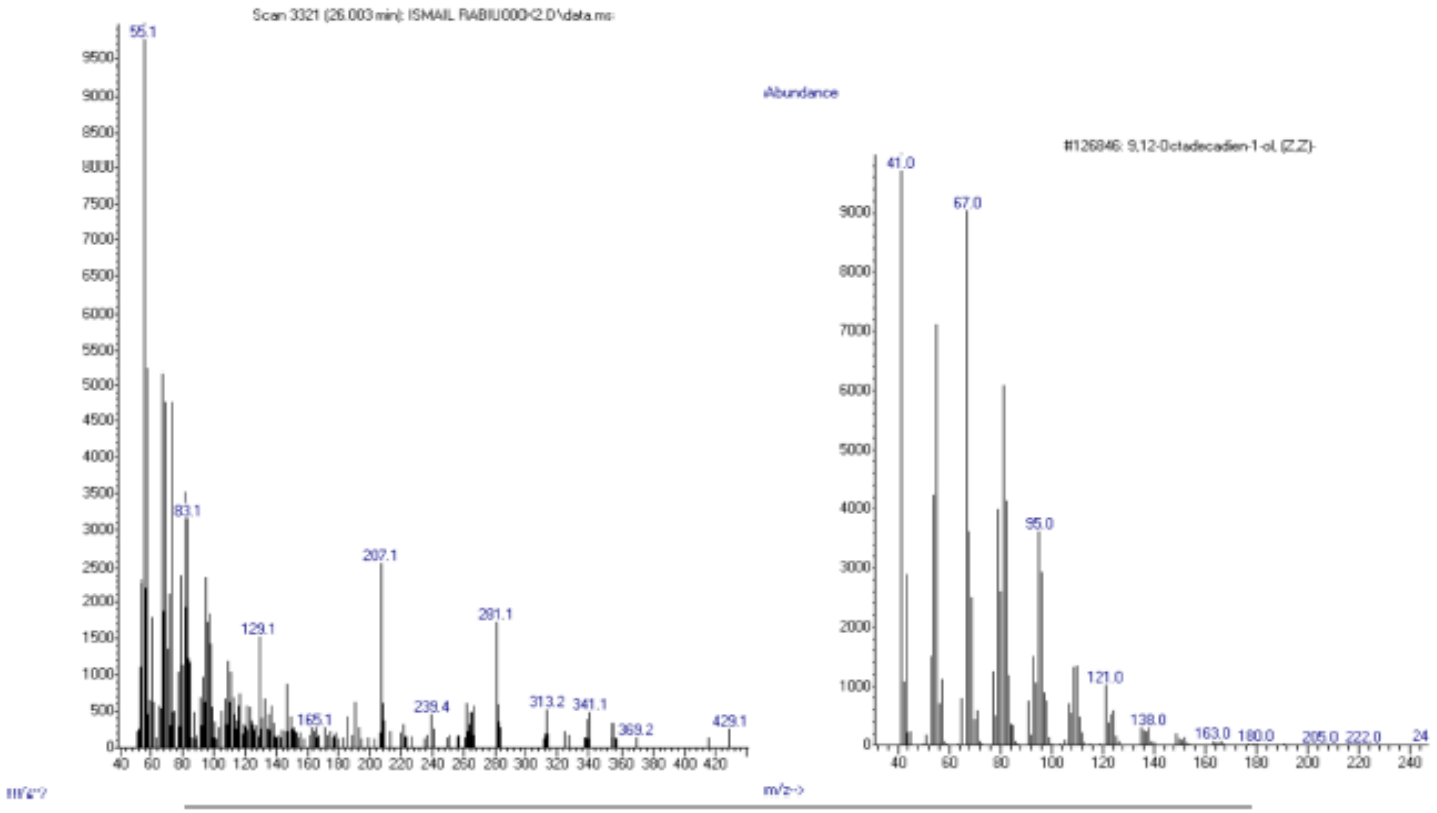

Figure 7 GCMS Chromatogram of the Methanol extracts of *C. esculenta* showing 9,12-Octadecadienoic acid

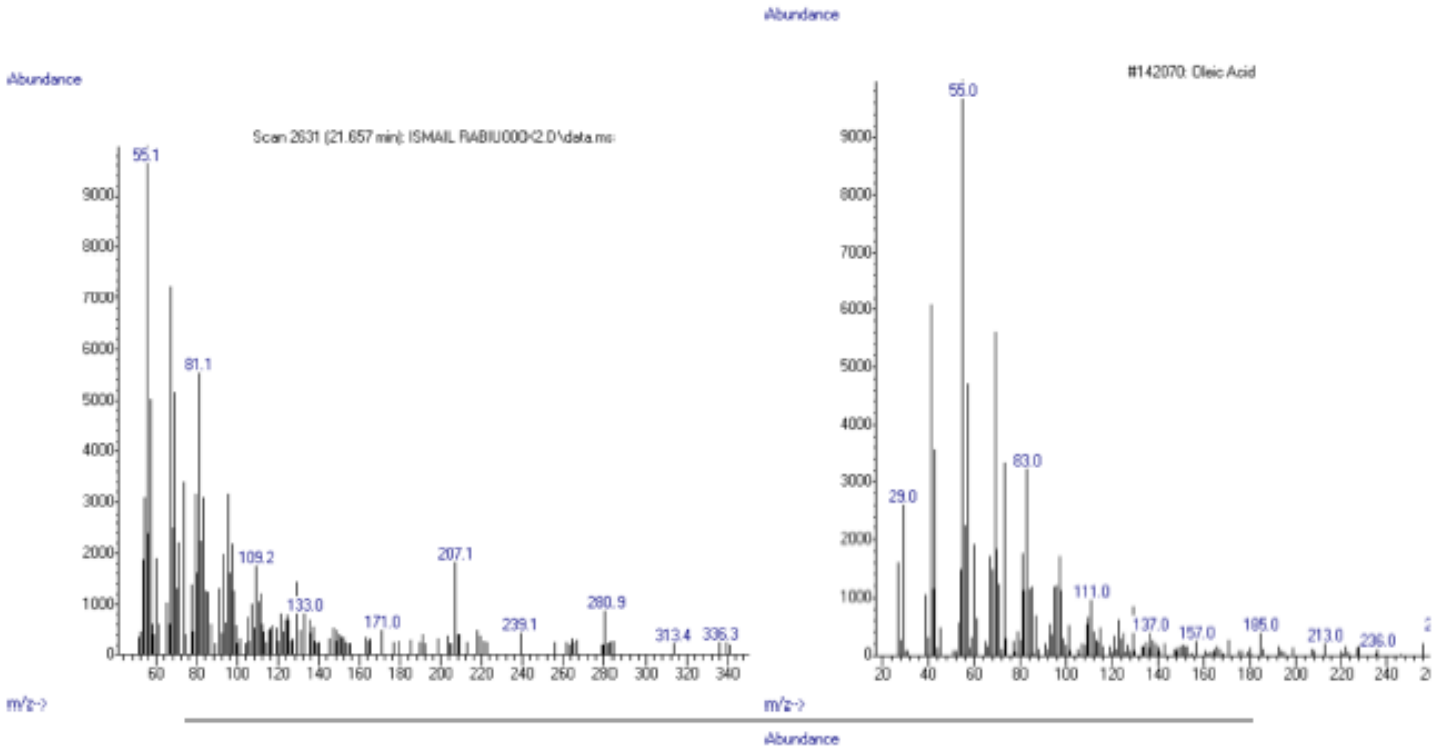

Figure 8 GCMS Chromatogram of the Methanol extracts of *C. esculenta* showing Palmitoleic acid

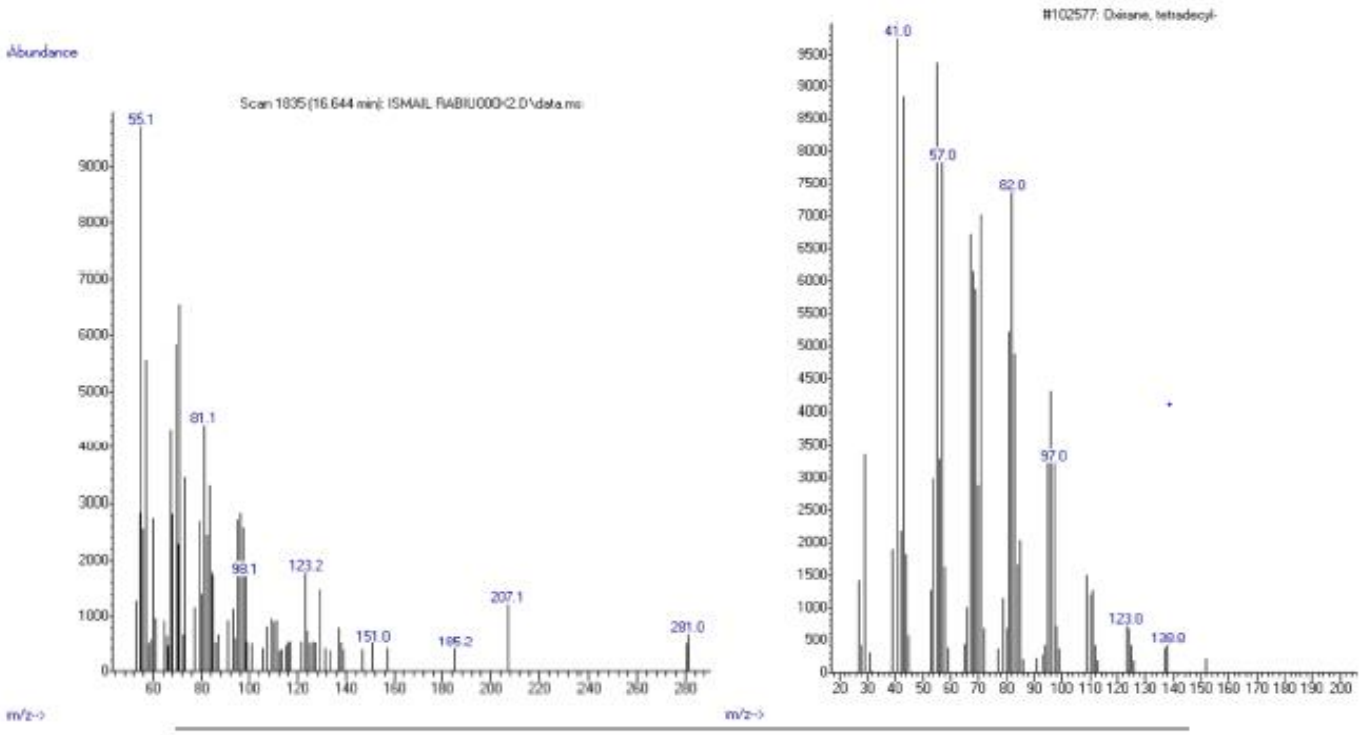

Figure 9 GCMS Chromatogram of the Methanol extracts of *C. esculenta* showing Oxirane

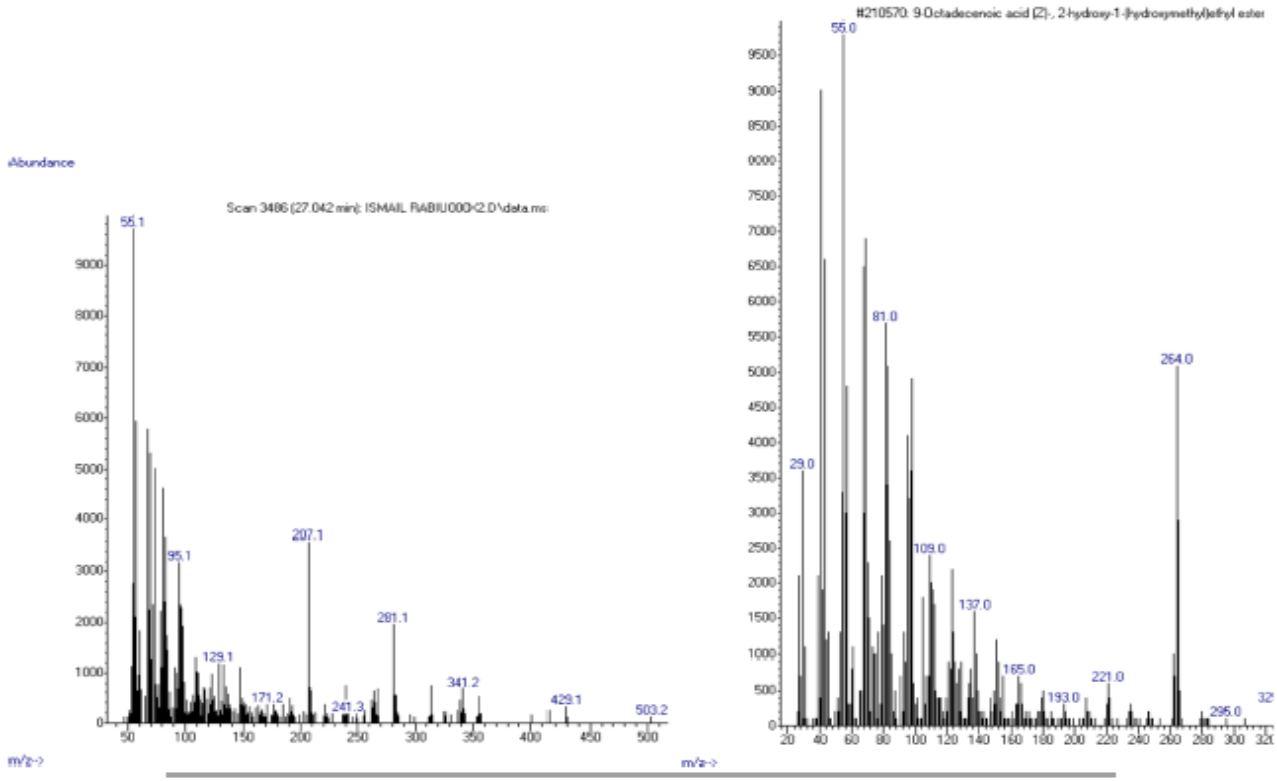

Figure 10 GCMS Chromatogram of the Methanol extracts of *C. esculenta* showing 2-hydroxy-1-(hydroxymethyl) ethyl ester

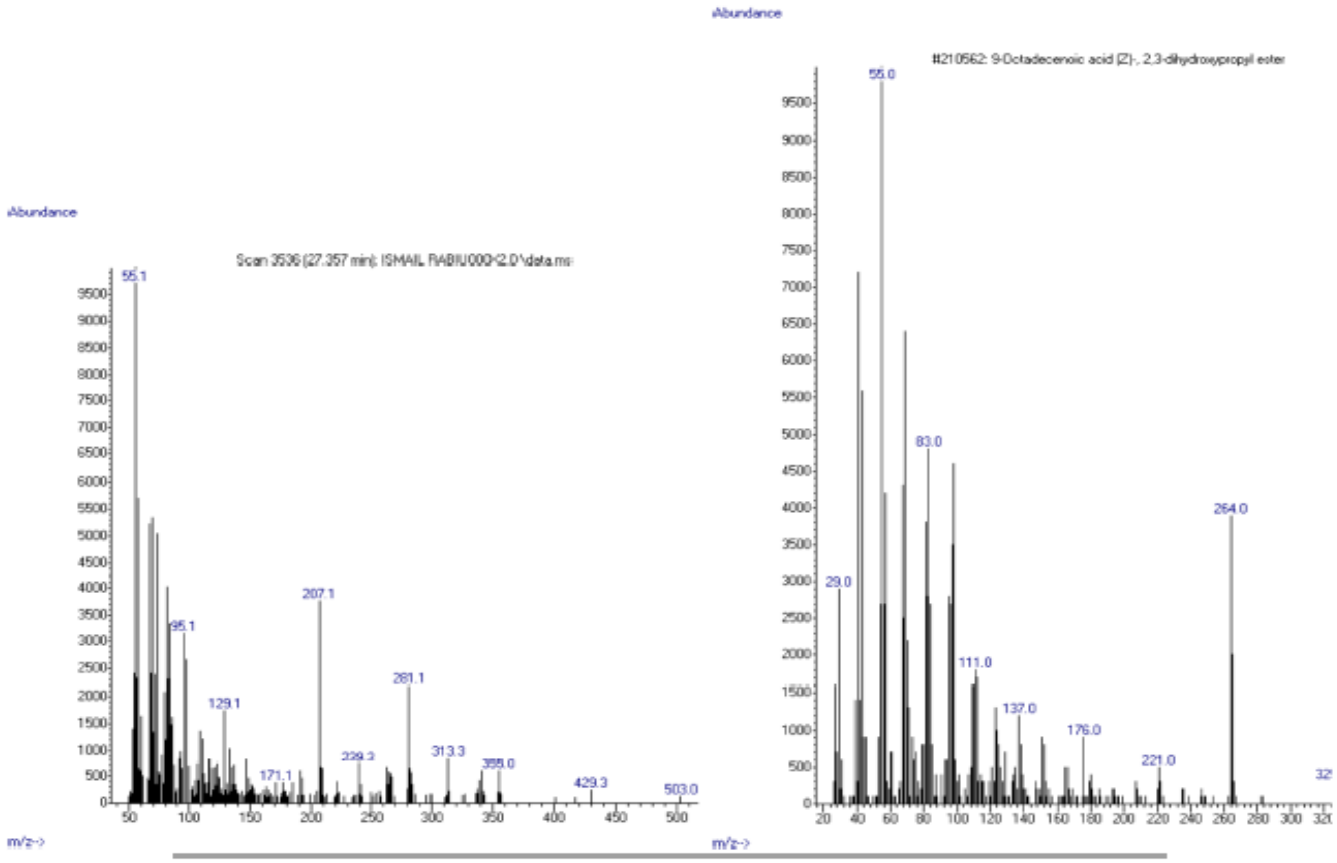

Figure 11 GCMS Chromatogram of the Methanol extracts of *C. esculenta* showing 2,3-dihydroxypropyl ester

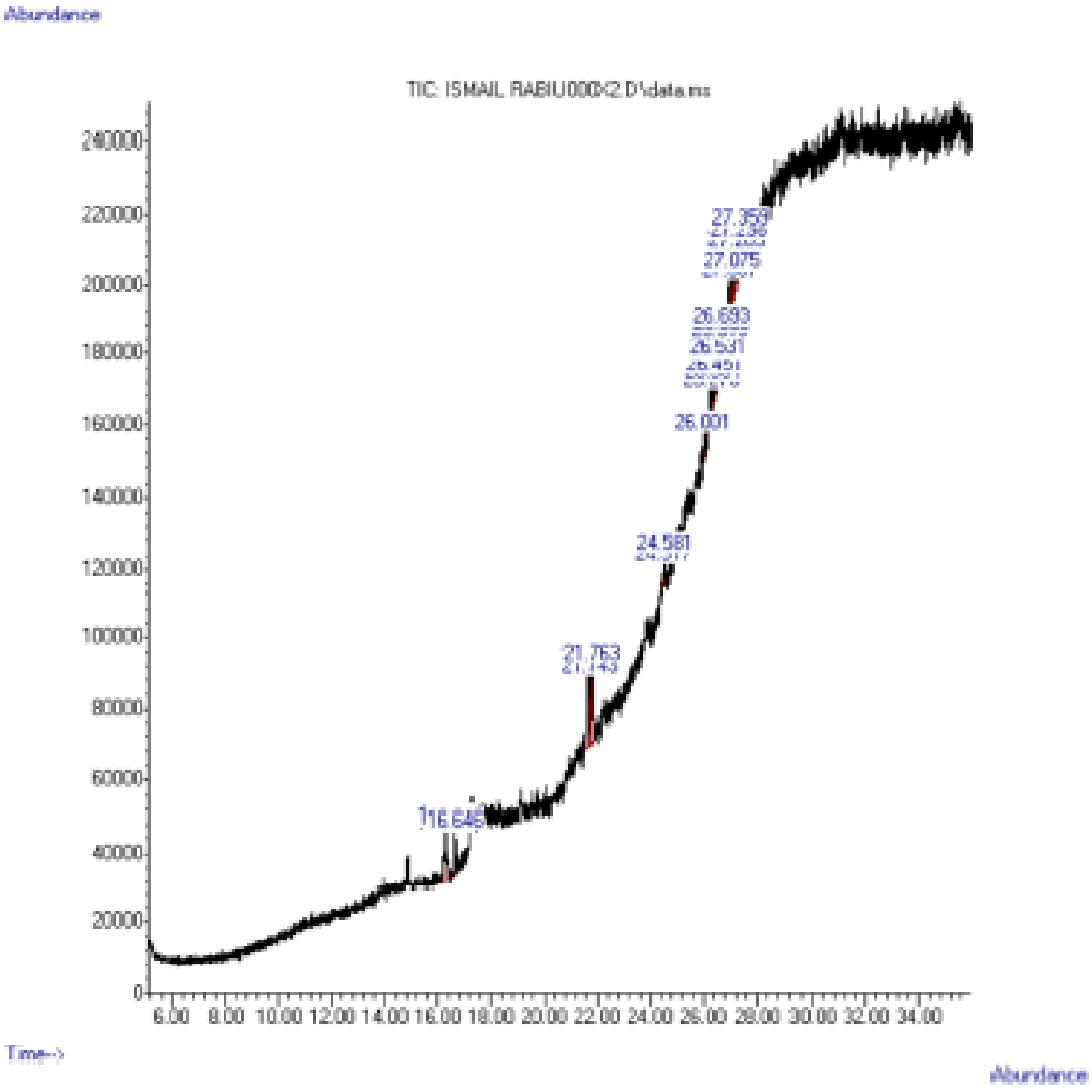

Figure 12 GCMS Chromatogram of the Methanol extracts of *M. esculanta* Showing the various phytoconstituents

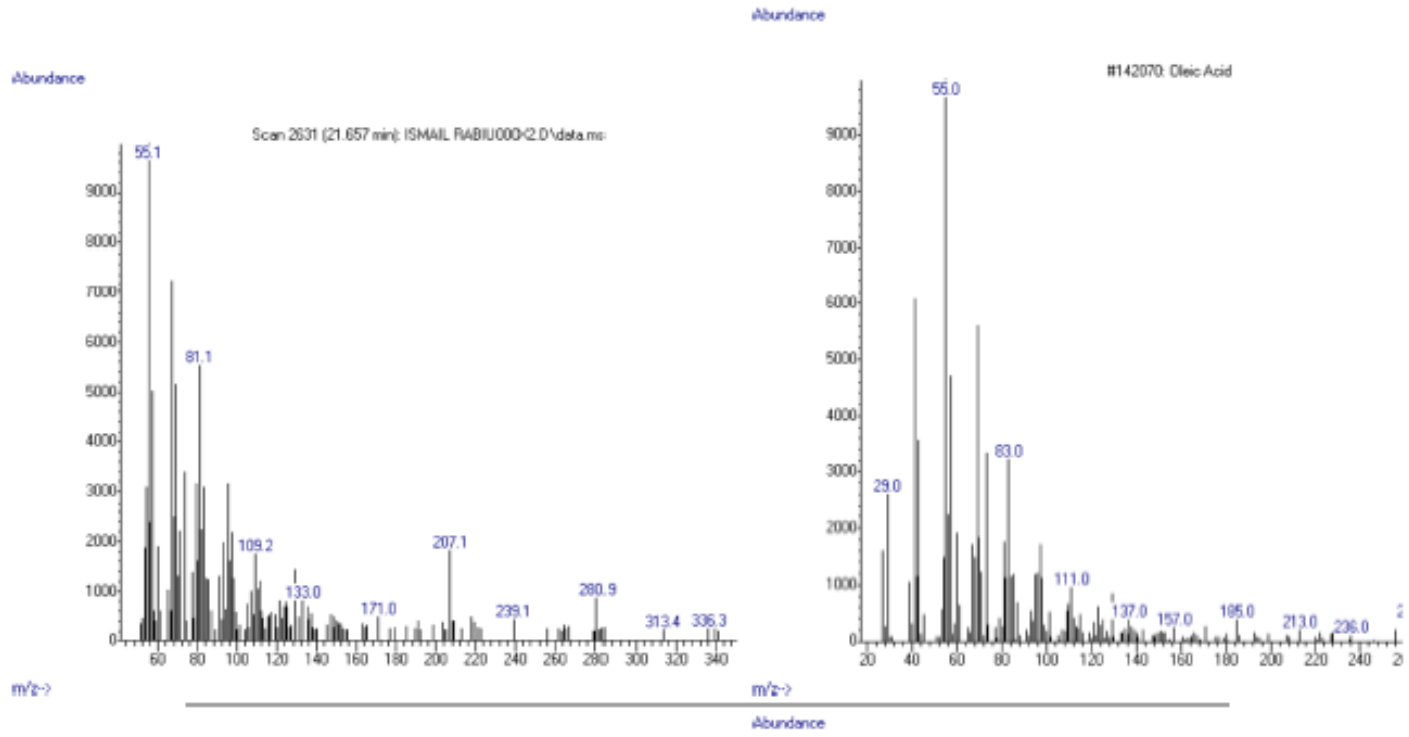

Figure 13 GCMS Chromatogram of the Methanol extracts of *M. esculanta* showing Oleic Acid

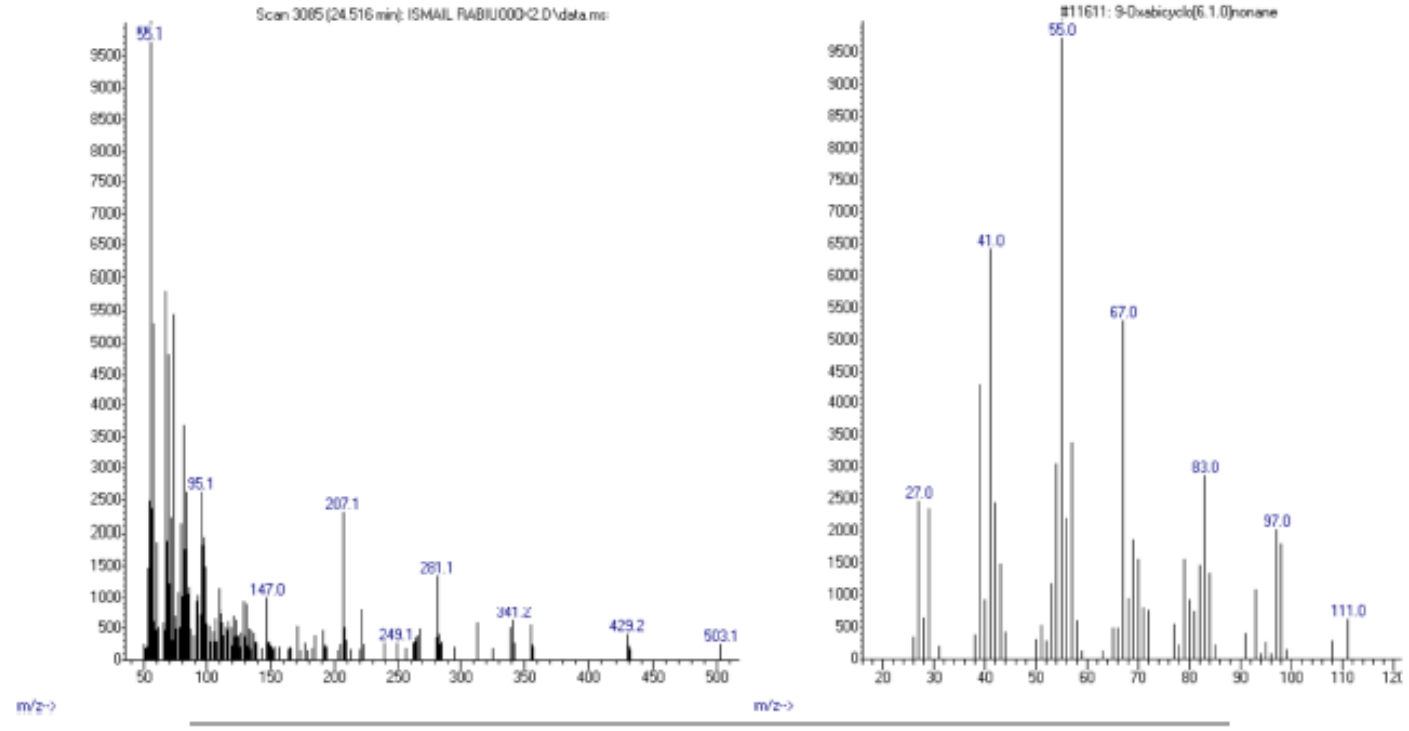

Figure 14 GCMS Chromatogram of the Methanol extracts of *M. esculanta* showing 9-Oxabicyclo [6.1.0] nonane

Table 4 Gas Chromatography-Mass spectroscopy analysis of the Methanol extracts of *C. esculenta*

Peak no.	Retention time (min)	% composition by area	Compound name
1	16.230	6.28	9,12-Octadecadienoic acid
2	16.351	5.15	Palmitoleic acid
3	16.646	4.80	Oxirane
4	21.659	13.26	Cyclotetracosane
5	21.743	3.19	9,12-Octadecadienoic acid (Z, Z)
6	21.763	12.95	9,12-Octadecadienal
7	24.517	1.84	9-Oxabicyclo [6.1.0] nonane
8	24.581	0.58	E-8-Hexadecen-1-ol acetate
9	26.001	0.77	Propyleneglycol monoleate
10	26.220	2.62	2-Methyl-Z, Z-3,13-octadecadienol
11	26.278	1.08	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate
12	26.364	2.16	2-3-dihydroxypropyl ester
13	26.451	2.16	9-Octadecenal
14	26.510	1.68	2,3-dihydroxypropyl ester
15	26.531	0.96	Butyl 9.cis.,11.trans.-octadecadienoate
16	26.616	4.17	1,3,12-Nonadecatriene
17	26.655	1.10	2,3-dihydroxypropyl ester
18	26.893	3.29	2-hydroxy-1-(hydroxymethyl)ethyl ester
19	26.919	11.50	Cyclopropaneoctanal
20	27.007	4.07	6-Octadecenoic acid,
21	27.040	2.04	2,3-dihydroxypropyl ester
22	27.075	1.46	2-hydroxy-1-(hydroxymethyl)ethyl ester
23	27.138	6.11	2,3-dihydroxypropyl ester
24	27.265	3.92	2,3-dihy droxypropyl ester
25	27.296	1.32	9,12-Octadecadien-1-ol,
26	27.359	2.05	2,3-dihydroxypropyl ester

Table 5 Gas Chromatography Mass spectroscopy (GCMS) profile of the Methanol extracts of *M. esculanta*

Peak no.	Retention time (min)	(%) composition by area	Compound name
1	6.914	0.14	Carbonic acid, tetradecyl vinyl ester
2	9.929	0.22	Thiophene
3	11.038	3.41	N-propyl decyl ether
4	11.396	0.62	Cyclododecanol, 1-ethenyl-
5	12.081	0.52	undecyl ester
6	12.226	0.08	7-Hexadecenal,
7	13.759	1.09	Pentadecanoic acid, 14-methyl ester
8	13.888	1.04	Hexadecanoic acid, methyl ester

9	14.548	2.00	3-Trifluoroacetoxypentadecane
10	14.738	0.82	Isopropyl palmitate
11	14.846	0.79	N-Hexadecanoic acid
12	15.241	3.50	7,11-Hexadecadienal
13	15.941	0.43	Ethylidenecycloheptane
14	16.250	6.66	methyl ester 13-Octadecenoic acid, methyl ester
15	16.351	5.57	trans-13-Octadecenoic acid, methyl ester
16	17.009	42.30	9,12-Octadecadienoic acid
17	21.112	0.67	9-Oxabicyclo [6.1.0] nonane,
18	21.676	1.77	1, E-11, Z-13-Octadecatriene
19	22.137	0.35	9,12-Octadecadienoyl chloride,
20	22.432	0.28	9-Oxabicyclo [6.1.0] nonane
21	22.770	1.24	9-Methyl-Z, Z-10,12-hexadecadien-1-ol acetate
22	24.330	-0.73	Cyclononasiloxane, octadecamethyl
23	25.016	5.63	Oleic Acid
24	25.556	0.72	9,12-Octadecadien-1-ol,
25	26.445	1.16	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-
26	27.156	2.36	Geranyl vinyl ether
27	27.748	0.58	3-Octyne, 6-methyl-
28	28.574	1.55	7,10-Hexadecadienoic acid, methyl ester
29	28.891	1.18	Oleic Acid
30	30.215	6.12	3,4-Octadiene, 7-methyl-
31	30.680	1.86	1,3,12-Nonadecatriene
32	31.160	1.81	Linoelaidic acid
33	32.131	2.81	9,12-Octadecadienal
34	34.425	0.79	9,12-Octadecadienal
35	34.448	0.65	9-Octadecenoic acid (Z)-, 2,3-dihy droxypropyl ester

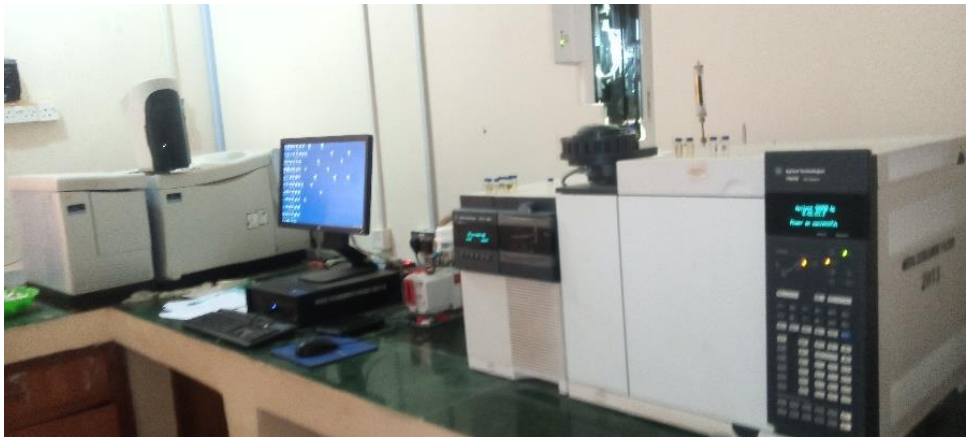

Figure 15 Gas Chromatography-Mass Spectroscopy Machine

Figure 15 presents a picture of the GCMS machine used for the analysis. Medicinal plants are known to contain many different varieties of low molecular mass compounds capable of producing certain pharmacological action when consumed (M et al., 2010).

Palmitoleic acid have been reported to have some fungicidal and bactericidal effect with high activity against multidrug-resistant bacteria. These fatty acids (linolenic, myristic, linoleic, myristic, linoleic, stearic, palmitic, and oleic acids), have proven effective against a wide range of bacterial pathogens with a high potential for the development of therapeutic agents (Nakade et al., 2013; Rawat and Gupta, 2011).

5. CONCLUSION

The results clearly indicate *M. esculanta* extracts as having a higher yield (Aqueous 26.7%; Methanol 13.3%) as compared to that of *C. esculenta* (Aqueous 16.0%; Methanol 8.2%), with a significant difference observed between the yield of the two plants extracts. The extracts have a different texture and chocking smell, with the methanol extracts having a greenish-black colour while the aqueous was having a reddish-brown colour. In both the methanol and aqueous extracts, different bioactive compounds were identified. These compounds possess antibacterial and antioxidant activity.

TLC analysis, reveals different bands from each of the extracts. The GCMS analysis of the methanolic extracts was identified as having 26 and 35 different compounds for *C. esculenta* and *M. esculanta* respectively, separated by their various peak numbers. Compounds such as 2-3-dihydroxypropyl ester, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12-Octadecadienoic acid, and Palmitoleic acid are the major constituents of the *C. esculenta* extracts, while *M. esculanta* extracts were observed to contain 9,12-Octadecadienal, Hexadecanoic acid, 9-Oxabicyclo [6.1.0] nonane, and Oleic acid, as the most abundant component.

The constituents of the leaf extracts and their level of activity recorded, showcase the pharmacological significance and could explain the use of these plants in infectious disease containment as claimed by traditional herbalists. This showcases the possibility of better chemotherapeutic outcome in the treatment of infections. Owing to their bioactivity, along with the high potential of the plants, there is the need to test these compounds against a wide range of pathogens including both fungal, viral, and bacterial pathogens.

Recommendation

Further studies should as well focus on finding the synergistic activity of the two plants (*M. esculanta,* and *C. esculenta*) when mixed together.

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Authors Contribution

Conceptualization; IR, MY, Methodology; IR, MY, Software; IR, Validation; IR, MY, AM, Formal analysis; IR, MY, AM, Investigation; IR, MY, AM, Resources; IR, MY, AM, Data Curation; IR, MY, Writing - Original Draft; IR, Writing - Review & Editing; IR, MY, AM, Visualization; IR, MY, AM, Supervision; IR, MY, Project administration; IR

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for collection & experimentation.

Data and materials availability

All data associated with this study are present in the paper.

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